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Approaches and Constraints to Identification and Quantitation of Asbestos Fibers

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Asbestos fibers, members of complex crystal–chemical systems, possess some range in characteristic properties. Identification of fibers requires morphological, structural, and chemical data. Most current work centers on identification of single, sublight-microscopic fibers present as contaminants in a range of media. Constraints encountered in the analysis of such materials are: sample preparation of the differing media; level of fiber exposure (contamination); presence (kind and amount) of other particles; tissue types and their different preparation techniques; homogeneity of samples and their preparations; use of proper instrumental technique; time required for analysis, and quantitation.

The Asbestos Minerals

The naturally occurring silicate fibers which are commercially used as "asbestos" include the minerals, amosite, anthophyllite, chrysotile, crocidolite, and tremolite *(1)*. Unlike any number of laboratory-prepared materials, which may be synthesized to yield a specific chemical composition and set of physical properties according to preparation, asbestos minerals are themselves variable representatives of more complex crystal–chemical systems. For example, amosite is a mineral term used to describe a long flexible fiber of commercial value in the mineral series cummingtonite–grunerite *(2)*. As mineral phases become more magnesium-rich in this series, containing greater than 70 mole-% magnesium, the structure changes from monoclinic to orthorhombic symmetry. This new material, with slightly different properties, is given the mineral name anthophyllite (another amphibole asbestos type). Therefore, the term amosite reflects a mineralogically complex material which may range in both chemical and physical properties. Although the asbestos minerals do exhibit variation from this standpoint, they possess sufficiently individual properties to permit identification *(3–5)*.

Most asbestos work today is carried out on the sublight-microscopic level, so that characteristics which are observable or measurable by electron beam instrumentation are used as standard identification criteria. Fiber morphology, selected-area electron diffraction pattern, and chemical analysis are used to identify a fiber uniquely *(3)*. These criteria have been used in many studies requiring identification of single asbestos fibers. However, occasionally, where occupational circumstances are encountered, a number of other instrumental techniques may be used for fiber characterization *(4)*. Here, large samples may be analyzed by any number of standard instrumental techniques. The basic requirement in these instances is bulk sample or many fibers (Table 1).

Asbestos Fiber Analysis: Basic Problem

The biological effects associated with the inhalation and/or ingestion of asbestos fiber are well documented. Many recent articles have summarized these observations with much literature documentation *(4)*. For tissues ob-

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Table 1. Possible instrumentation for fiber analysis and constraints.

Instruments	Starting material and information obtained	Constraints
Single particle, large Light microscopy Bright field illumination Phase contrast Polarized light	Paraffin sections,[a] stained or unstained; ashed or unashed; bulk extracts in immersion oils Dust distribution in tissues; very limited morphology and size-distribution; crystallinity, limited indices of refraction and optical properties; identification of some phases	Size and resolution limited; small, particles not detectable; light visible particles seen only; overlapping properties of phases; change of "standard" optical properties with reduced size
Single particle, small Electron beam instruments Transmission electron microscope Selected-area electron diffraction Electron microprobe analyzer Scanning electron microscope with energy dispersive x-ray detector	Paraffin sections[a] followed by carbon extraction; bulk extracts mounted on appropriate substrates Single particle analysis; diffraction data of groups of particles; number, morphology, size distribution, relative crystallinity of dusts, structure and chemical composition of particles; unique identification of phases.	Area scanned is small and may not be representative; large number of particles required for statistical evaluation; sample preparation and scanning is time-consuming; instrumentation and interpretation expertise required
Many particles X-ray diffraction Diffractometer Film technique	Paraffin sections[a] may be used if sufficient dust present; bulk extracts more useful Bulk analyses are obtained (mixed phases and x-ray patterns); crystallinity of phases; average size distribution of phases; identification of crystalline phases	Mixed dusts yield x-ray patterns difficult to interpret; small particles yield diffuse patterns; body salts confound analyses; line-broadened spectra common; extraction technique may alter phases or produce an artifact dust population
Other techniques Infrared spectroscopy (IR) Differential thermal analysis (DTA) Emission spectroscopy (ES) X-ray fluorescence Atomic absorption spectroscopy (AAS) Wet chemical analysis (WCA)	Bulk materials required in all cases, usually "pure" samples, free of organic contaminants; samples required range in size from micrograms to grams. Entire particle populations are analyzed simultaneously; structural and molecular makeup for identification of phases; thermal reactions for characterization; bulk and trace chemical analyses of dusts	IR and DTA data are ambiguous, difficult to interpret uniquely; bulk chemistry by ES, AAS, WCA is destructive; chemical data difficult to assign to individual phases; nature of trace metal phases unknown.

[a] For fibers in tissues.

tained from workmen occupationally exposed to asbestos fiber, the techniques required for tissue preparation and analytical procedures are many and varied. Here the amount of fiber per unit mass of tissue is high, making analysis far easier as compared to tissues obtained from individuals who were not occupationally exposed to fiber *(5)*. This observation focuses on the basic problem involving fiber analysis in a matrix of other materials. It may be likened to the electronic problem involving pulse analysis with an unfavorable "signal-to-noise" ratio. For example, we have examined digested lung tissues obtained from asbestos workmen exposed to amphibole fiber. Some 100 to 500 uncoated fibers may be observed on each electron microscope grid field (an area of some 100 × 100 μm). The ratio of fiber to other particle debris is high, making location and analysis an easy step. Similar preparations of lung tissues obtained from people in the general population, those not occupationally exposed to asbestos, have demonstrated that there are orders of magnitude less fiber per comparable area. More important, in these individuals there also occurs other inorganic particulate matter which must be examined during the electron microscope scan. Therefore, much time is spent visually

scanning the "noise" while searching for the fiber "signal". This problem is further amplified when tissues other than lung are examined for their asbestos content. Even in asbestos workmen, the extrapulmonary organs contain what appears to be magnitudes less fiber as compared to their lung tissues. Again, an unfavorable signal-noise ratio is encountered. Extrapolation of these findings to the general population would suggest that studies involving the search for asbestos fiber in these extrapulmonary organs would be extremely difficult and time-consuming. Therefore, the basic problem of fiber analysis involves the search for the particle itself. Fiber analysis in most cases is now readily accomplished.

Matrix Materials: Different Levels of "Noise"

The analysis of asbestos fiber in air, water, or tissue samples each has its own relative level of difficulty. For example, the search for asbestos fiber captured on an air filter is far less difficult than for a similar search on an identical membrane substrate for a water sample. Water samples, depending on the source, may contain myriad other particles, both of organic and inorganic origin, which possess a fibrous morphology. The signal-noise ratio in such a case is severe, and much time must be spent in distinguishing among the various fibrous particles present in the assemblage. This essentially means stopping an EM scan to perform selected area electron diffraction on each fiber observed. Microchemical analysis on each fiber is almost mandatory, especially on those structurally determined to be amphiboles. The time involved in analysis of such samples is enormous.

Preparation Techniques for Fiber Analysis

Various media have been sampled to determine their asbestos fiber content. These are: gases, commonly air *(6)*; liquids (for example, parenteral drugs) *(7)*; and solids (for example, human tissues) *(4)*. Each of these samples consists of fibrous particles in different media which must be prepared for instrumental analysis. For example, air and water samples tend to consist of particulate matter entrapped on the surface of some filter substrate. This material is either organic membrane or polycarbonate, which must be removed before instrumental analysis. Depending on the source of the sample, the particle population may be extremely complex. Tissue analysis may be more complicated, in that the particle population tends to be more varied, consisting of exogenous particles and endogenous salts. Tissues themselves react differently to the range of preparation treatments. The level of exposure varies, resulting in different particle densities. Even the preparation technique itself may create particle artifacts. In this latter case, bulk digestion of tissues has in the past resulted in chemical and physical degradation of some types of asbestos fiber *(8)*. Here a residue population may result which does not reflect the original population. Even with these difficulties, techniques are now available which permit examination of either single histologic sections *(3)* or bulk tissues *(9)*. Careful preparation will yield particle populations without alteration. One such technique makes use of common laundry bleach (sodium hypochlorite) as the principle chemical reagent. We have slightly modified this technique with great success. We are now able to routinely reduce any tissue for particle analysis. The state of the art is such that now virtually any gas, liquid or solid material may be prepared for examination for its asbestos fiber content.

Instrumental Technique

A number of instrumental techniques may be used for the detection, identification and characterization of asbestos fibers. These include: light microscopy, x-ray diffractometry, electron beam instrumentation including probe techniques, infrared spectroscopy, differential thermal analysis, emission and atomic absorption spectroscopy. These various instruments, the quantities of material required for analysis, the information obtained with such instrumentation, and the limitations attached to the use of such instruments are given in Table 1.

From the information provided in Table 1, one can envisage that a number of instruments may be used for asbestos fiber characterization; each yields different information and possesses a number of limitations to its use. For example, although electron beam instruments are superior for the identification and characterization of single asbestos fibers *(9)*, the area searched is generally small and may not be represen-

tative of the total sample. Also, much time is required for each analysis; this limits the number of particles, as well as the number of samples, which may be analyzed. Not withstanding these difficulties, electron beam instrumentation is recommended for fiber analysis *(4)*.

General Considerations

It is now possible routinely to analyze air, liquids, or tissue or other solid materials for their asbestos fiber content. Instrumentation required for such analysis is a function of many interrelated factors which have been described elsewhere *(4)*. Basically, the fibers of biological interest are sublight-microscopic in size and, in general, require electron beam instrumentation for identification and characterization. Samples of particulates suspended in air or liquids are readily filtered onto an appropriate substrate which may be in turn prepared for electron microscopy. Tissue samples or any other solid substance may be reduced and prepared for electron beam instrumentation as well. These techniques are now established and in the literature. These had been developed to reduce the creation of artifacts in the particle population.

The major constraint which now exists is the signal-to-noise ratio. Asbestos fiber may coexist with any number of other inorganic particulates, which may be fibrous in morphology. This problem is lessened in areas where asbestos contamination is high. For example, samples obtained at the emission source of fiber or within the work site environment, or from tissues of occupationally exposed workmen, are heavily laden with fiber and do not present such problems. As samples are obtained further from emission sources the signal decreases, and the relative background "noise" increases. As this unfavorable background becomes prominent, the time for analysis increases proportionately. Because of the training required for the individual performing the analysis, the cost of maintaining such instrumentation, the time required for such analysis, the number of such analyses are small while the cost is high.

The quantitation of asbestos fiber in samples is only at the beginning stages of work. Air samples, by virtue of being less contaminated with other particles, produce the most suitable sample for quantitation. At the present time, the quantitation of asbestos in water samples may be done only with great effort. This requires morphological, structural, and chemical characterization of single fibers for just counting purposes. A characterization of 100 fibers from such a sample, depending on fiber density in relation to other particles, may take several weeks of work. Quantitation of asbestos fiber in tissue is presently being worked on in this laboratory. The quantitation of asbestos fiber should be done by an automated technique.

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REFERENCES

1. Speil, S., and Leineweber, J. P. Asbestos minerals in modern technology. Environ. Res. **2:** 166 (1969).
2. Deer, W. A., Howie, R. A., and Zussman, J. Rock Forming Minerals, Vol. 2. Chain Silicates. Longmans, Green, London, 1967, p. 234.
3. Langer, A. M., and Pooley, F. D. Identification of single asbestos fibers in human tissues. *In: Proceedings International Conference on Biological Effects of Asbestos*, Lyon, France, 2–6 Oct. 1972, P. Bogovski, J. C. Gilson, Timbrell, and J. C. Wagner, Eds., IARC, Lyon, 1973, p. 119.
4. Langer, A. M., et al. Identification of asbestos in human tissues. J. Occup. Med. **15:** 287. (1973).
5. Ehrenreich, T., et al. Identification and characterization of pulmonary dust burden in pneumoconiosis. Ann. Clin. Lab. Sci. **3:** 118 (1973).
6. Selikoff, I. J., Nicholson, W. J., and Langer, A. M. Asbestos air pollution. Arch. Environ. Health **25:** 1 (1972).
7. Nicholson, W. J., Maggiore, C. J., and Selikoff, I. J. Asbestos contamination of perenteral drugs. Science **177** 171. (1972).
8. Langer, A. M., Selikoff, I. J., and Sastre, A. Chrysotile asbestos in the lungs of persons in New York City. Arch. Environ. Health **22:** 348 (1971).
9. Jaunarajs, K. L., and Liebling, R. S. The digestion of lung tissue for mineral dust recovery. J. Amer. Ind. Hyg. Assoc. **1:** 535 (1972).